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Histochemistry of the Mucous Cells in the Skin of *Pseudobagrus fulvidraco* and *Leiocassis nitidus* (Bagridae, Siluriformes)

Yong-Ho Kim, Chung-Lyul Lee and Sung-Ju Jye-Gal*

Department of Biology, College of Natural Science, Kunsan National University, Kunsan 573-701, Korea
*Department of Clinical Pathology, College of Wonkwang Health Science, Iksan 570-750, Korea

Skin mucous cells in the three regions of the body in *Pseudobagrus fulvidaraco* and *Leiocassius nitidus* were investigated using three histochemical methods (PAS, AB-PAS and HID). In the two species, components of mucous cell were not distinguished in each region, but presented a differences in position and type, size, and count in all regions. Mucous cells of *P. fulvidraco* were located in two layers, a superficial epithelial cell and the space within alarm substance cells (ASCs). Mucous cells of *L. nitidus* were situated only in the superficial epithelial cell layer. The size of mucous cells in the ASC layer were larger than those of the superficial epithelial cells for all measured values: diameter, length, width, and area. In the superficial epithelial cell layer, the average number of mucous cells within the three regions did not differ significantly between species by unpaired T-test, but the average number of those within two cell layers of *P. fulvidraco* had a similarity at each region by paired T-test. The dismilarity in dorsal and ventral regions in average number of ASCs between *P. fulvidraco* and *L. niditus* was confirmed by unpaired T-test.

Key words : Histochemistry, mucous cell, skin, *Pseudobagrus fulvidraco, Leiocassis nitidus,* Bagridae

Introduction

The fishes inhabit at unique environment as it is called water, a accommodation of the epidermis that is direct in contact with their environment have been maintain stability to the interior milieu in water (Matoltsy and Bereiter-Hahn, 1986). The skin of the fishes protects themselves and its inside condition maintain uniformly from outside surrounding environment (Park *et al*., 1995a) and the main function of the integument of vertebrates is to control and to protect the organism from the environment (Matoltsy and Bereiter-Hahn, 1986). Most skin of the fishes is covered with scales and it does protect itself from mechanical wounds and predators (Lagler *et al*., 1977; Moyle and Cech, 1996). However, some species have no scale, but they secrete mucus from specialized mucous cells in epidermis to the outside surface of skin continually (McKim *et al*., 1996). The secreted mucus constitutes the primary biological interface between fish and the aqueous environment and plays an important role of protection against a mechanical injury, friction reducing properties, possibly in ionoregulation, and first barrier against infection, because of having defence factors in the mucus, such as immunoglobulin, lysozyme and lectin (Iger *et al*., 1994; Strüssmann *et al*., 1994; Moon, 1995a; Sabóia-Moraes *et al*., 1996; Burkhardt-Holm *et al*., 1997; Ottesen and Olafsen, 1997; Quiniou *et al*., 1998; Burkhardt-Holm *et al*., 2000; Zhang *et al*., 2000).

Pseudobagrus fulvidraco and *Leiocassis nitidus* appertaining to the family Bagridae are species having no scale and keep a lot of mucus to a exterior of epidermis from a mucous cell. Mucous cells can be classified by various stain methods (Whitear, 1986; Mittal *et al*., 1994) based on qualitative difference of a chemical component in both interspecies and intraspecies (Ottesen and Olafsen, 1997).

Already many study of mucous cell at epidermis of fishes have been performed between male and female (Irving, 1996) or genera (Gona, 1979; Satō, 1979; Singh and Mittal, 1990). On the other hand, nowadays *P. fulvidraco* and *L. nitidus* were reappraised the taxonomic position by Lee (1988), which is having very interesting aspects, although it was decided into different genus clearly based on the their osteological characters.

The aim of this study is to make a check on components, location, count and size of mucous cells in epidermis between two species by light−microscope.

Materials and Methods

In this study, six specimens of *P. fulvidraco* (102.0～175.2 mm) and ten of *L. nitidus* (161.7～171.4 mm) were collected at Guiam, Buyeo−up Buyeo−gun, Chungcheongnam−do, Korea in 2000. All specimens were fixed in 10% neutral buffer formalin at the living condition immediately and each specimen were dissected to obtaining about 5×5 mm of skin fragments from three region, that is dorsal, lateral, and ventral respectively. Dissected fragments carried out the successive course of dehydration in ethanol for paraffin embedding, clearing in xylene, and paraffin infiltration in paraffin wax. Embedded samples were sectioned at thickness of 5 μm by rotary microtome. To identify a ingredients of mucous cell was stained with three methods, periodic acid Schiff (PAS), alcian blue at pH 2.5−periodic acid Schiff (AB−PAS), and high iron diamine (HID) at pH 2.5 of alcian blue (McManus, 1946; Mowry, 1963; Spicer, 1965). The section was chosen randomly from each fish and the number of mucous cells and alarm substance cells (ASCs) counted in a captured image (the dimension is about 75,000 μm^2, n = 40) by Image plus pro 4.0 (400×). Average number of mucous cells and ASCs obtaining from random area were compared by T−test.

Results

1. Component of mucous cell

We confirmed that *P. fulvidraco* and *L. nitudus* contained neutral mucous cells in dorsal region by PAS stain method (Fig. 1). Consequently, in the three regions of *P. fulvidraco* and *L. nitidus*, it was not discovered a different components in their mucous cells. Mucous cells in two species, in general, contained a mixture of neutral and acidic by AB−PAS (purple in colour) (Fig. 2), and sulfonic and sialic components by HID (brown in colour) (Fig. 3), but a few mucous cells showed the only neutral or acidic and sulfonic or sialic component. However, most mucous cells had sulfonic component than sialic in two species relatively (Fig. 3).

2. Size of mucous cell

Mucous cells of ASC layer of epidermis in *P. fulvidraco* were larger than those within superficial epithelial cell layer for several measured values : diameter and length, over two times; width, over three; area, over six (Table 1). And it appeared that the mucous cells of *L. nitidus* was some larger than those of *P. fulvidraco* of the superficial epithelial cell layer relatively (Table 1).

3. Location and type of mucous cell

In *P. fulvidraco*, There are two types of mucous cell in three regions; the one presented in the superficial epithelial cell layer and the other did in the space of ASC layer of epidermis (Figs. 1A−3A), in *L. nitidus*, however, mucous cells situated only in the superficial epithelial cell layer in three regions (Figs. 1B−3B). In *P. fulvidraco* and *L. nitidus*, some mucous cells in the superficial epithelial cell layer showed the extended shape horizontally, and sometimes others appeared the

Table 1. Measurements of the mucous cell (n = 40) in the skin of *Pseudobagrus fulvidraco* and *Leiocassis nitidus* (mean±S.D.)

Division	*P. fulvidraco*		*L. nitidus*
Cell layer	superficial	alarm	superficial
Diameter (μm)	7.1±2.0	17.9±3.2	8.5±2.1
Length (μm)	9.5±3.2	22.6±5.2	10.6±2.9
Width (μm)	6.0±1.9	18.9±7.3	8.2±2.2
Area (μm^2)	44.1±23.0	266.5±96.9	64.1±33.0

extended shape vertically. However, mucous cells in the ASC layer of epidermis showed almost round shape. Mucous cells of *L. nitidus* of superficial epithelial cell layer were denser than those of *P. fulvidraco*. So, in the ASC layer of epidermis of *P. funvidraco*, it was very sparsely distributed.

4. Count of alarm substance cells (ASCs)

The average number of ASCs between *P. fulvidraco* and *L. nitidus* in three regions showed similarity each other (in *P. fulvidraco* : dorsal and lateral, $P<0.05$; lateral and ventral, $P<0.05$; dorsal and ventral, $P<0.05$ and in *L. nitidus* : dorsal and lateral, $P<0.05$; lateral and ventral, $P<0.05$; dorsal and ventral, $P<0.05$) (Fig. 4A). In the intraspecies the average number of ASCs did not have a similarity among all regions.

But in the interspecies, there was not similarity at dorsal and ventral regions except lateral : dorsal, $P>0.05$; lateral, $P<0.05$; ventral, $P>0.05$ (Fig. 4B). At dorsal and ventral region, the average number of ASCs of *L. nitidus* was more than those of *P. fulvidraco* (Fig. 4B).

5. Count of mucous cell

Three regions of between two species and two kinds of mucous cells within *P. fulvidraco* were compared by T−test. First, in the superficial epithelial cell layer the average number of mucous

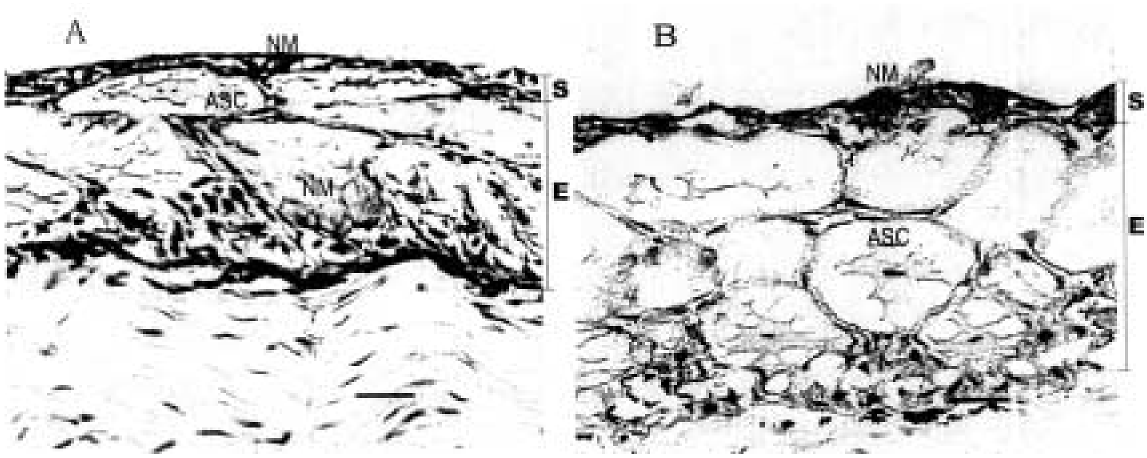

Fig. 1. Periodic acid Schiff stained sections of dorsal skin of *Pseudobagrus fulvidraco* (A) and *Leiocassis nitidus* (B). Scales indicate 20 μm. ASC, alarm substance cell; E, epidermis; NM, neutral mucous cell; S, superficial epithelial cell layer.

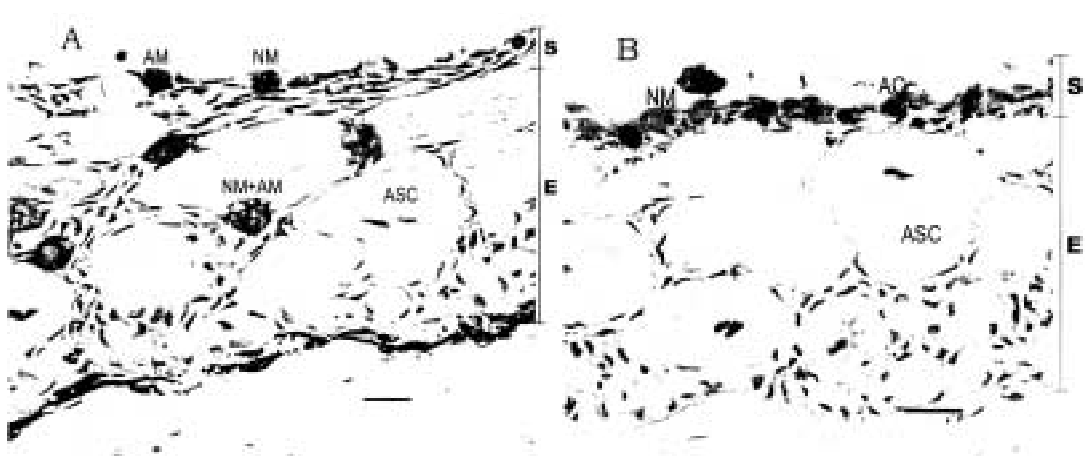

Fig. 2. Alcian blue (pH 2.5)−periodic acid Schiff stained sections of lateral skin of *Pseudobagrus fulvidraco* (A) and *Leiocassis nitidus* (B). Scales indicate 20 μm. ASC, alarm substance cell; E, epidermis; NM, neutral mucous cell; S, superficial epithelial cell layer.

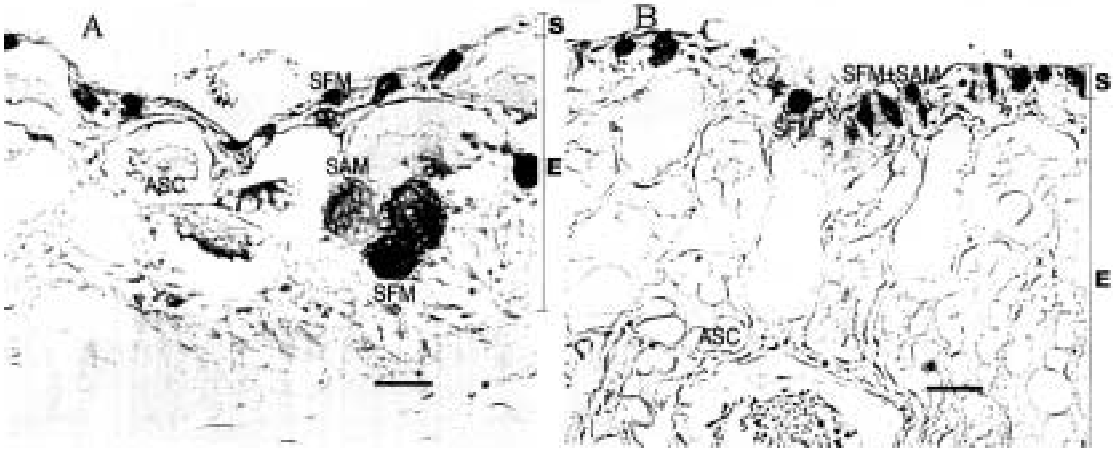

Fig. 3. High iron diamine (alcian blue pH 2.5) stained sections of ventral skin of *Pseudobagrus fulvidraco* (A) and *Leiocassis nitidus* (B). Scales indicate 20 mm. ASC, alarm substance cell; E, epidermis; S, superficial epithelial cell layer; SAM, sialomucous cell; SAM+SFM, mixture of sialo and sulfomucous cell; SFM, sulfomucous cell.

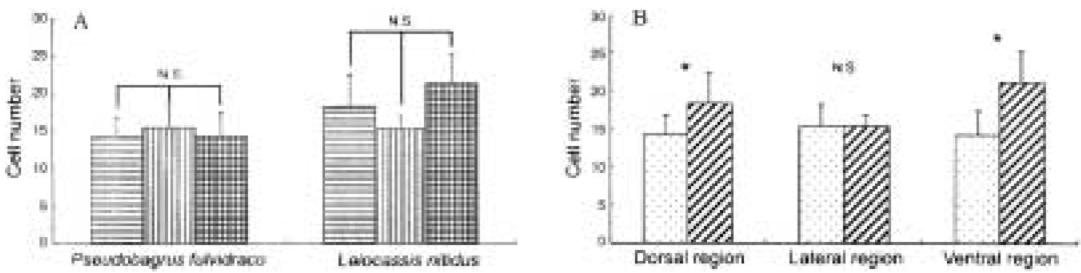

Fig. 4. Count of alarm substance cell of *Pseudobagrus fulvidraco* and *Leiocassis nitidus* (A : ▤, dorsal; ▥, lateral; ▦, ventral) in three regions and at three regions of skin in between two species (B : ▒, *P. fulvidraco*; ▨, *L. nitidus*). Bars represent mean+S.D.. Differences of three regions at each species and between *P. fulvidraco* and *L. nitidus* are signification at : *, $P < 0.05$; N.S., $P > 0.05$ (T-test).

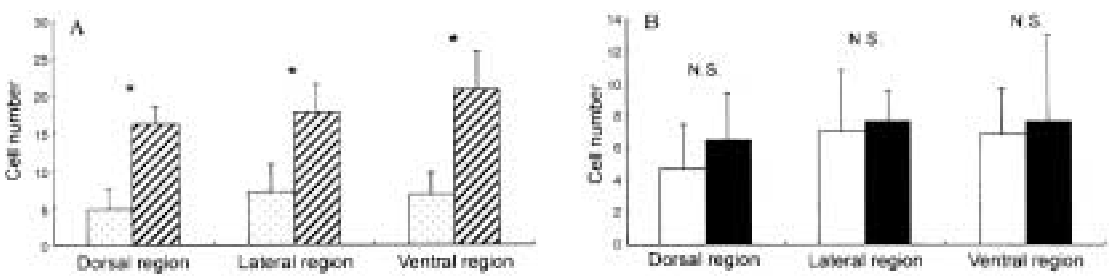

Fig. 5. Counts of mucous cell of superficial epithelial cell layer of *Pseudobagrus fulvidraco* (▒) and *Leiocassis nitidus* (▨) (A) and superficial epithelial cell layer (□) and alarm substance cell of epidermis (■) of *P. fulvidraco* (B). Differences of three regions in between two species and different types within *P. fulvidraco* are signification at : *, $P < 0.05$; N.S., $P > 0.05$ (T-test).

cells had not a similarity between *P. fulvidraco* and *L. nitidus* at the three regions : dorsal, $P < 0.05$; lateral, $P < 0.05$; ventral, $P < 0.05$ (Fig. 5A), but it of *L. nitidus* was much more than it of *P. fulvidraco* relatively.

Second, mucous cell in the ASC layer in the epidermis in *P. fulvidraco* had a similarity at the three regions for it in the superficial epithelial cell layer : dorsal, $P > 0.05$; lateral, $P > 0.05$; ventral, $P > 0.05$ (Fig. 5B). As a results, *P. fulvidraco* largely differed from *L. nitidus* in the average number of mucous cells at all regions.

Discussion

The present study was performed for investigation of ingredient, size, location, type and count of mucous cell in the skin between *P. fulvidraco* and *L. nitidus* of belonging to the family Bargridae by histochemical methods.

Whitear (1986) mentioned that each species of fishes usually secrete a different type of mucus according the nature of mucous cell. *Betta splendens* has a only neutral mucous cells and *Macropodus opercularis* showed acid but non-sulphated ones, while *Colisa lalia* and a species of the genus *Trichogaster* had mixed populations of mucous cells differing in the proportion with neutral glycoprotein in skin (Gona, 1979). Moon (1995b) reported that in the gill epithelium the guppy (*Poecilia reticulatus*) also has the single mucous cell contained either neutral or acid glycoproteins alone or in combination. However, the components of the mucous cell was changed from sulfomucin to sialomucin by a influence of seawater (Fletcher *et al*., 1976; Gona, 1979; Moon, 1995b). *L. nitidus* inhabits at the freshwater having a effect upon seawater (Kim, 1997), if so, this species may be have a lot of sialomucin than *P. fulvidraco*. Whitear (1986) mentioned that taxonomy is no guide to the type of mucus secreted. However, A differences of mucous cell may be due to an adaptation to the vastly diverse habitats of a teleost fishes and a bottom-dewellers produce, with a few exceptions, exclusively sulphated glycoproteins and a sulphation confers increased charge on the mucins, which may affect the rheological properties of the mucus (Ottesen and Olafsen, 1997), while a species with a pelagic habitat produce merely a carboxylated mucus (Whitear, 1986). But *P. fulvidraco* and *L. nitidus* have a lot of sulphated mucous cells than sialoid (Fig. 3) relatively. It is proved that they may be a bottom-dweller indirectly. The sulphated mucus may, in particular, have a role of protection from an abrasive environment for benetic species (Mittal *et al*., 1994). When designing a cultivating unit for two species, such fact should be taken in to consideration.

Mucous cells of *P. fulvidraco* are distributed at two locations, which are the superficial epithelial cell and ASC layer of epidermis, but *L. nitidus* was only one, that is the superficial epithelial cell layer. According to a species, some species have only one type or more two type of mucous cells (Satō, 1978). Such differences of the position and type may be due to have a different habitat between two species. Generally *P. fulvidraco* dwells in a mid-stream and downstream of river with the slow speed of current, but *L. nitidus* live lower area of river where somewhat influenced by seawater (Kim, 1997). Park *et al.* (1995b) mentioned that when the freshwater eel (*Anguilla japonica*) of a adapted condition in freshwater came in contact with a seawater, a vacuoles with agranule and space of among the cell within epidermis increase at the inside of epidermis, while such happenings didn't appear between epithelial cells and mucous cells. But *P. fulvidraco* and *L. nitidus* have a similar habit as a bottom-dweller (Lee, 1990; Kim and Kang, 1993; Kim, 1997). And in a alteration of osmotic balance mucous cell must be take charge of a role of protection against a wound or abrasion (Moon, 1995a). If so, because of *L. nitidus* is adapted to often changing water environment, according to the habitat, it may be have this position. We are considered that *L. nitidus* had a unique arrangement of mucous cell separately from *P. fulvidraco* (Figs. 1-3).

In *Cyprinus carpio* var. *communis* the presence of a large number of mucous cells in the outer regions of the epidermis suggests that the overall production of mucus in this fish is very high and this may be as an adaptation in relation to its peculiar bottom-scooping habits for increasing efficiency in the fish keeping its surface clean (Singh and Mittal, 1990). In mucous cells within the superficial epithelial cell layer of *P. fulvidraco* and *L. nitidus*, ones of the latter is more than ones of the former. From that point view, *L. nitidus* may be considered as an adaptation to their bottom feeding habits than *P. fulvidraco*.

In *P. fulvidraco*, mucous cells in ASC layer were larger than that within the layer of other epidermis cells and it was presented the two position, which are the superficial epithelial cell and ASC layer of epidermis. Sighn and Mittal (1990) reported that mucous cells of *C. carpio* var. *communis* were located mainly in the outer region of the epidermis, *Labeo calbasu* and *Cirrhina mrigala*, in surface layer, and *Catla catla* was found in the superficial and under deeper layers of the epidermis. Also a certain freshwater catfish (*Ictalurus punctatus*) had a same position with mucous cell of *P. fulvidraco* (Quiniou *et al*., 1998).

The number of ASCs in *P. fulvidraco* was fewer than those of *L. nitidus* in the dorsal and ventral region. In a comparative study of the epidermis of the craps, Singh and Mittal (1990) reported that numerical relation of ASC and mucous cell is a interdependence, and high density of ASCs may compensate for the smaller and sparser mucous cells in providing and effective defense mechanism. Therefore, *P. fulvidraco* and *L. nitidus* of dissimilarity of ASCs in number may be relative the whole number of mucous cells. And, in two regions of *P. fulvidraco*, relationship of mucous cells may be suggest that its ASC layer at epidermis supplement a few mucous cells in the superficial epithelial cell layer for providing a lacking mucus production.

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동자개(*Pseudobagrus fulvidraco*)와 밀자개(*Leiocassis nitidus*)에 대한 피부점액세포의 조직화학 (Bagridae, Siluriformes)

김 용 호 · 이 충 렬 · 제갈승주*

군산대학교 자연과학대학 생물학과, *원광보건대학 임상병리과

동자개과 (Bagridae) 어류에 속하는 동자개 (*P. fulvidraco*)와 밀자개 (*L. nitidus*)의 3부위 (등면, 측면 그리고 배면)의 피부 점액세포를 광학현미경하에 조직화학적인 방법으로 조사했다. 각 종의 피부에 존재하는 점액세포를 PAS, AB-PAS 그리고 HID의 염색방법을 통해 비교해 본 결과, 두 종간에 점액세포의 성분적 차이를 발견할 수 없었고, 그러나 조사 부위에서 점액세포의 위치, 형태, 크기 그리고 수에 있어서 차이가 존재했다. 동자개의 점액세포들은 편평상피세포층과 상피의 alarm substance cells (ASCs)층에 존재했다. 그러나 밀자개의 점액세포들은 편평상피세포층에만 존재했다. 상피의 ASC 층에 존재하는 점액세포는 편평상피세포층에 존재하는 것보다 상대적으로 더 컸다. 편평상피세포층에서 두 종간 각 부위의 점액세포의 평균수는 이분산 T-test에 의해 인정되었고, 등분산 T-test에 의한 동자개의 두 층의 점액세포간의 평균수는 차이가 인정되지 않았다. 그리고 이분산 T-test에 의한 동자개와 밀자개 상피의 ASC 평균수는 등면과 배면에서만 차이가 인정되었다.